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Natural polymorphism affecting learning and memory in *Drosophila*

Frederic Mery*†, Amsale T. Belay‡, Anthony K.-C. So‡, Marla B. Sokolowski‡, and Tadeusz J. Kawecki*§

*Department of Biology, University of Fribourg, Chemin du Musée 10, CH 1700 Fribourg, Switzerland; †Laboratory for Evolution, Genome and Speciation, Centre National de la Recherche Scientifique, Avenue de la Terrasse, F 91198 Gif sur Yvette, France; and ‡Department of Biology, University of Toronto, 3359 Mississauga Road, Mississauga, ON, Canada L5L 1C6

Knowing which genes contribute to natural variation in learning and memory would help us understand how differences in these cognitive traits evolve among populations and species. We show that a natural polymorphism at the *foraging* (*for*) locus, which encodes a cGMP-dependent protein kinase (PKG), affects associative olfactory learning in *Drosophila melanogaster*. In an assay that tests the ability to associate an odor with mechanical shock, flies homozygous for one natural allelic variant of this gene (for^R) showed better short-term but poorer long-term memory than flies homozygous for another natural allele (for^s). The for^s allele is characterized by reduced PKG activity. We showed that for^R-like levels of both short-term learning and long-term memory can be induced in for^s flies by selectively increasing the level of PKG in the mushroom bodies, which are centers of olfactory learning in the fly brain. Thus, the natural polymorphism at *for* may mediate an evolutionary tradeoff between short- and long-term memory. The respective strengths of learning performance of the two genotypes seem coadapted with their effects on foraging behavior: for^R flies move more between food patches and so could particularly benefit from fast learning, whereas for^s flies are more sedentary, which should favor good long-term memory.

Learning and memory allow an individual to develop an adaptive behavioral response to a novel situation, even one never encountered in the evolutionary past of the species. The ability to learn may thus be regarded as one of the more remarkable products of biological evolution. Yet, our understanding of how changes in learning ability evolve remains rudimentary (1). In particular, we know almost nothing about the genetic and molecular nature of heritable variation in learning performance. This variation is the raw material of evolution. Thus, knowing which genes contribute to natural variation in learning ability would help us understand how differences in learning ability and memory evolve among populations and species. It would also offer insights into the tradeoffs constraining the evolution of improved learning performance (1–3).

That natural populations harbor heritable variation affecting learning and memory has been demonstrated by artificial selection experiments, which succeeded in elevating learning performance in rats (4), blowflies (5), and *Drosophila* (6, 7). However, the genes underlying these experimentally induced evolutionary changes have not been identified. Mutants with major defects in learning or memory, a number of which are known in *Drosophila* (8–11), *Caenorhabditis elegans* (12, 13), and rodents (14, 15), tell us little about how genes contribute to the normal range of individual differences in learning abilities within a species. So far, the only polymorphic genes thought to contribute to natural variation in learning performance, in any species, have been recently identified through polymorphism-association studies in humans (16, 17). However, given the obvious constraints on human research, it will be difficult to study the evolutionary forces acting on these allelic variants and maintaining the polymorphisms. More insights into those forces could be gained from studying genes contributing to variation in learning performance in natural populations of model organisms, such as *Drosophila*. As a candidate for such a gene, we focused on an already well characterized natural polymorphism.

The gene *foraging* (*for*), which encodes a cGMP-dependent protein kinase (PKG), occurs in two common variants (alleles) in natural populations of *Drosophila melanogaster*. Flies carrying the so-called ''rover'' allele (for^R) show higher PKG activities and move more while feeding than those homozygous for the ''sitter'' allele (for^s) (18–20). Rovers are also more responsive to sucrose and show slower habituation of this response than sitters (21). Under laboratory conditions, the evolutionary success of rovers vs. sitters is affected by population density (22) and may be maintained by negative frequency-dependent selection (23). Both density and frequency dependence are likely to contribute to the maintenance of this polymorphism in nature. The implication of mammalian PKG in neurotransmission, synaptic plasticity, and motor learning (24, 25) makes this polymorphism a promising candidate for the identification of natural alleles that affect learning and memory. Here we show that this natural polymorphism affects associative learning: flies carrying the natural allele for^R show better short-term learning response but poorer long-term memory than flies homozygous for the other natural allele, for^s. We verify these antagonistic effects with mutants and transgenes and show they are mediated by localized expression of *for* in the mushroom bodies, known to be centers of olfactory learning in the fly's brain.

Results

We used an aversive olfactory conditioning assay (3) to compare the learning performance of flies homozygous for the natural rover (for^R) and sitter (for^s) alleles. Flies were conditioned to associate one of two odors (octanol or methylcyclohexanol) with mechanical shock and were subsequently tested for choice between these odors in a T-maze (Fig. 1*A*). Fifteen minutes after a single conditioning trial, the for^R strain showed a significantly stronger avoidance of the odor previously associated with shock than the for^s strain (Fig. 1*B*). A similar pattern was observed in flies assayed 15 min after a spaced conditioning protocol, which consisted of five rounds of conditioning separated by 20-min rest

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Abbreviation: PKG, cGMP-dependent protein kinase.

§To whom correspondence should be sent at the present address: Department of Ecology and Evolution, University of Lausanne, Le Biophore, CH 1015 Lausanne, Switzerland. E-mail: tadeusz.kawecki@unil.ch.

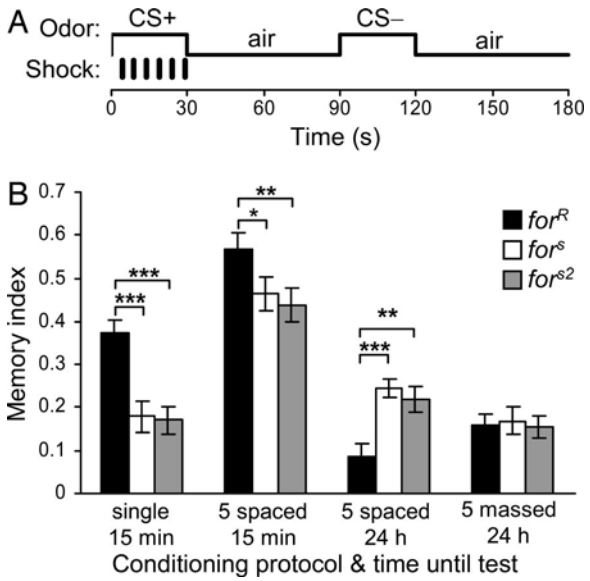

Fig. 1. *for* and learning performance. (*A*) The time course of one conditioning cycle. Flies were exposed to one odorant (CS+) and simultaneously subject to mechanical shocks. After a 60-s pause, during which they received clean air, they were exposed to another odor (CS−) without shocks. Octanol and methylcyclohexanol were used as odorants. Conditioning consisted of a single cycle, of five cycles separated by 20-min intervals (spaced protocol), or of five cycles immediately following one another (massed protocol). Memory tests were carried out in a T-maze 15 min or 24 h after the end of conditioning. (*B*) Mean memory index (±SEM) of flies homozygous for natural rover (for^R) and sitter alleles (for^s) and for sitter mutant (for^{s2}) generated on the for^R genetic background. For each assay, two separate experiments were carried out several generations apart, with a total of 16–20 replicate values of memory index per assay and strain. Significance was tested with Tukey's test following an ANOVA controlling for block effect: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

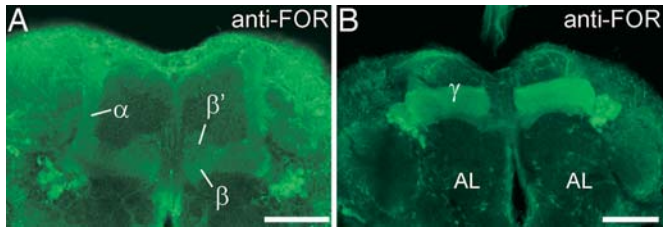

Fig. 2. The expression of FOR in the mushroom bodies, centers of olfactory learning in the *Drosophila* brain. (*A*) FOR is expressed in the α/β mushroom body neurons, which project to the vertically oriented α lobes (α) and the medially oriented β lobes (β) of the mushroom bodies, and in the α'/β' neurons, which project to α' and β' lobes. Only the β' lobe is distinctly visible; the α' lobe is intertwined with the α lobe. (*B*) FOR is also expressed in γ neurons that project to medially oriented γ lobes (γ). The images are 3D reconstruction of confocal sections spanning the mushroom bodies where dorsal is up (and tilted back in *B*). The antennal lobes are marked (AL) to show the relative orientation of the brain. The flies in the image are from the for^R strain, but no difference in the spatial expression of FOR between for^R and for^s flies has been detected. (Scale bar: 50 μm.)

intervals (Fig. 1*B*). The strains did not differ in their response to the odors in the absence of conditioning [supporting information (SI) Fig. 4], which makes it unlikely that the differences in learning performance are because of differences in olfactory perception. Thus, the for^R strain showed a stronger short-term response to conditioning.

Subsequently, we studied the effect of *for* alleles on consolidated memory. *Drosophila* have two mechanistically distinct forms of consolidated memory, which can last for >24 h: anesthesia-resistant and long-term memory (10, 26). Long-term memory is more stable, requires protein synthesis and, in classical aversive conditioning, forms only after repeated conditioning cycles separated with rest intervals (spaced protocol) (3, 10, 26). In contrast, anesthesia-resistant memory does not depend on protein synthesis and can also form when conditioning is carried out without rest intervals (massed protocol) (10, 26). No difference between the strains was observed 24 h after a massed conditioning protocol (Fig. 1*B*), indicating no difference in anesthesia-resistant memory. In contrast, 24 h after the spaced conditioning protocol, the for^R strain showed weaker memory of the association between an odor and shock than for^s (Fig. 1*B*), a difference due to long-term memory. Thus, the natural polymorphism at *for* has antagonistic effects on different aspects of learning performance: compared with the for^R strain, for^s flies perform poorly shortly after conditioning but show better long-term memory retention.

The strains carrying the natural for^R and for^s alleles differ with respect to their genetic background at chromosome 2, where the *for* locus is located. To substantiate the role of *for* in causing the differences in memory reported above, we also assayed flies homozygous for a mutant sitter allele, for^{s2}, generated on a for^R genetic background (18, 21). These mutant flies are similar to the for^s flies in having both reduced PKG levels and sitter-like foraging behavior (18, 20). In all assays, the learning performance of the for^{s2} mutant flies was indistinguishable from flies homozygous for the natural allele for^s (Fig. 1*B*). Because the for^R and for^{s2} strains are isogenic except at the *for* locus, this demonstrates the differences in learning and memory are specific and localizable to *for*.

Most neuronal processes underlying associative olfactory learning in *Drosophila*, including memory formation and retrieval, occur in a paired neuropil structure in the brain called the mushroom bodies. The intrinsic neurons of the mushroom body (Kenyon cells) can be divided in three subsets based on the targets of their axonal projections, one subset project to the α and β lobes of the mushroom body, another to α' and β' lobes, and a third to γ lobes (10). Significantly, using immunostaining, we found that FOR is expressed in the mushroom bodies, including all three subsets of mushroom body neurons (Fig. 2). (FOR is also expressed in several clusters of neurons outside of the mushroom body; detailed analysis is reported in ref. 27.)

Although no differences in the spatial pattern of FOR expression between for^R and for^s flies have been detected (27), the heads of for^R flies show higher PKG activity than those of for^s and for^{s2} (18). Therefore, we tested whether for^R-like learning and memory performance may be induced by increasing the level of PKG in the mushroom bodies of for^s flies. The GAL4-UAS dual system is a standard *Drosophila* technique used to express a gene of interest in specific organs or tissues, the specificity being determined by the identity of the GAL4 enhancer-trap driver (28). We used three GAL4 drivers with expression in the mushroom bodies (30Y, c739, and 201Y; Fig. 3*A*) to drive expression of the UAS-*for*T2 transcript (18) in flies homozygous for for^s. These flies showed improved 15-min memory scores, similar to those observed in for^R flies and significantly higher than the controls (Fig. 3*B*). Concomitantly, their long-term memory was significantly reduced to, or below, the levels typical for for^R flies (Fig. 3*C*). All three GAL4 drivers were expressed in the mushroom body neurons projecting to the α/β lobes (although for 201Y the expression was weak), and c739 in particular seems to be specific to these neurons (Fig. 3*A*; see also refs. 29–31). Thus, expression of *for*T2 in the α/β mushroom body neurons is sufficient to induce for^R-like learning performance in for^s flies. These results suggest that the modulating effect of allelic variants of *for* on olfactory learning and memory occurs in the mushroom bodies, in particular in their α/β neurons.

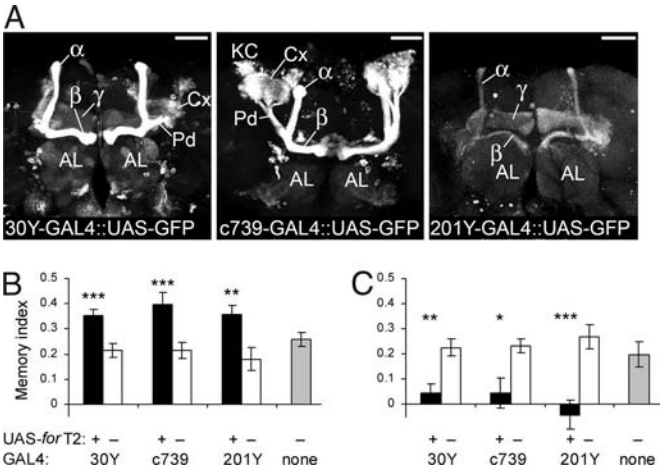

Fig. 3. GAL4-driven transgenic expression of *for* in the mushroom bodies of for^s flies improves short-term learning performance but reduces long-term memory. (*A*) Expression patterns of the GAL4 drivers in the fly brain, visualized with a mCD8 GFP reporter. AL, antennal lobes; α, β, α', β', and γ, mushroom body lobes; Pd, mushroom body peduncle; Cx, mushroom body calyx; KC, Kenyon cell bodies; the scale bar corresponds to 50 μm. All three drivers are expressed in the α and β lobes of the mushroom body. 30Y is also expressed in neurons projecting to α' and β' lobes (these lobes are located behind the α and β lobes; only α' is distinctly visible). 30Y and 201Y are also expressed in the γ lobes. (*B* and *C*) Mean memory index (±SEM) of flies expressing UAS-*for*T2 transcript under the control of each GAL4-driver (black bars); the flies carrying only the GAL4 element (white bars) or only the UAS-*for*T2 construct (gray bar) serve as controls. All flies had $w;for^s$ genetic background. (*B*) Fifteen-minute memory after a single conditioning trial. (*C*) 24 h memory after five spaced conditioning trials. Sample size, 10 replicate values of memory index per bar. The symbols indicate the significance of a contrast between a given GAL4 × UAS line and the two corresponding controls (GAL4 × $w;for^s$ and $w;for^s$ × UAS) in the ANOVA, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Discussion

Our results show that the rover/sitter polymorphism contributes to genetic variation in associative learning in natural populations of *Drosophila*. Furthermore, the effects of these alleles on learning performance shortly after conditioning and on long-term memory are antagonistic. Thus, variation in *for* may mediate an evolutionary tradeoff between short- and long-term memory. Finally, our results point to the mushroom bodies, which are centers of olfactory learning in the fly brain (10), as the spatial focus of the action of PKG (the enzyme encoded by *for*) on learning performance. In the following, we first place our findings in the context of what is known about the role of PKG in learning and memory and then discuss their ecological and evolutionary implications.

PKG in Neuronal Processes Underlying Learning and Memory. The cellular functions of PKG are poorly elucidated, and its downstream effectors, for the most part, are unknown. Mammalian PKG (also called cGKI) plays a role in synaptic plasticity (long-term potentiation and depression) and seems to act both pre- and postsynaptically as a downstream component of nitric oxide signaling (24). Mice deficient in cGKI are defective in a cerebellum-dependent motor-learning task (32), but their performance in hippocampus-dependent learning is apparently not affected (33). However, pharmacological potentiation of NO-cGMP signaling was reported to improve the performance of mice in a hippocampus-dependent learning task, the water maze (34), whereas pharmacological inhibition of PKG impaired memory retrieval in chickens (35). Finally, NO-cGMP signaling was recently shown to interact with a cAMP-dependent mechanism in long-term memory formation in crickets (36). Although pharmacological inhibition of PKG had no effect on memory in that study, it indicated that memory processes dependent on cGMP may run in parallel to processes mediated by other signaling pathways, like the *rut* adenylate cyclase-dependent memory trace (10, 29, 36).

Interactions between such parallel pathways might be responsible for the antagonistic effects of *for*-PKG on short- and long-term memory, which we report here. Yet, long-term memory is thought to form on the basis of short-term memory (with middle-term memory being an intermediate step) (10, 11), so one would rather expect them to be positively correlated. A positive correlation between short-term learning performance and long-term memory was observed in fly populations subject to selection for improved performance in an ecologically relevant oviposition learning task (37). The antagonistic effects of FOR on short-term learning performance and long-term memory we report here are thus unexpected and call for more research on the role of cGMP-dependent processed in memory formation.

Although we can only speculate about the mechanism by which *for*-PKG acts to modulate learning and memory, our findings clearly point to mushroom bodies as the spatial focus of its action. We found FOR expressed in all three subsets of mushroom body neurons (α/β, α'/β', and γ). However, transgenic expression of *for*T2 transcript restricted to the α/β neurons was sufficient to induce for^R-like pattern of short- and long-term memory in for^s flies. The α/β neurons play a central role in olfactory memory: memory retrieval relies on synaptic output from these neurons (30, 31), and the α lobes contain a long-term memory trace (38, 39). However, we cannot exclude a role of FOR in α'/β' and γ neurons, which have also been implicated in olfactory learning (29–31). In particular, the GAL4 driver 201Y shows only weak expression in the α/β neurons and apparently only in those that project to the cores of the α/β lobes; this line seems to express more strongly in the γ neurons (see also ref. 29). Yet, using it to drive the expression of *for*T2 had the same effect on learning performance as that using the other two driver lines. This implies either that a low level of *for*T2 expression in the α/β neurons is sufficient for the full effect on short- and long-term memory, or that *for*T2 expression in the γ neurons also contributes to this effect. Identification of GAL4 lines specific to α'/β' and γ neurons (30, 31) will help to resolve the effect of *for* expression in those neurons on memory formation and consolidation.

Ecological Significance of *for*'s Effects on Learning. There is a wealth of evidence for the ecological significance of learning in a variety of insects (1, 40–46). In *Drosophila*, experimental data suggest that larvae use learning to find food and avoid predators (47); oviposition substrate choice of females is modified by experience (7); and males learn to discriminate against heterospecific females (48) and to recognize unreceptive females of their own species (49), as well as refine their courtship behavior (50). Thus, even though our understanding of ecological aspects of learning in fruit flies is still rudimentary, there is evidence that it contributes to their fitness under natural conditions. This would explain why *Drosophila* are capable of learning, despite learning ability being a costly adaptation (2, 3, 51). But is the effect of *for* polymorphism on learning and memory ecologically relevant? The classical conditioning paradigm used in this paper allows us to control the amount of shock and odors received by the flies and to dissect the memory dynamics, but its relevance to situations in which *Drosophila* learn in nature is unclear. Nonetheless, different forms of olfactory learning, involving different contexts and stimuli, rely at least in part on the same genes and neural circuits (10, 52) and are affected by the same naturally occurring genetic variation (37). In accord with that notion, the *for* alleles also affect larval appetitive learning (53). Thus, it is

reasonable to expect that the learning and memory differences among *for* genotypes will affect their learning performance in nature and thus may contribute to natural selection on this polymorphism.

The extent to which learning ability is favored by natural selection and which aspects are favored should depend on the environment (1, 54). In particular, fast learning would be highly advantageous if the environment changed frequently within the lifetime of an individual, whereas good long-term memory would be particularly useful in more stable environments. Arguably, rover (for^R) flies are more prone to encounter different environments within their lifetime than sitter (for^s) flies; they spend less time feeding at one location, both as larvae and as adults, and are more likely to leave a patch of food in search of another one (18–20). It is thus tempting to speculate that the superior short-term learning performance of for^R flies and the good long-term memory of for^s flies form elements of complex rover and sitter evolutionary strategies, respectively adapted to variable and constant environments. However, one might also argue that rover flies would benefit from good long-term memory if they revisit places visited previously; resolving this argument would require a better understanding of *Drosophila* field ecology than we have currently. In the absence of evidence, it is more parsimonious to regard the antagonistic effects of the *for* alleles on short-term learning and long-term memory as a mechanistic consequence of the role of PKG in neuronal processes. As discussed above, too little is known about this role to understand the mechanism of this antagonism. It is also not clear whether this antagonism is typical for natural allelic variants, leading to a strong tradeoff between short- and long-term memory. The pattern of genetic correlations among different memory phases in natural gene pools has not been investigated, except for one study where both short-term learning rate and long-term memory improved in response to selection on learning performance in an ecologically relevant task (37).

Whether they form part of coadapted alternative strategies or are mechanistic consequences of differences in PKG activity, the learning and long-term memory differences among *for* genotypes are likely to contribute to natural selection on the allelic variants of *for* polymorphism. However, in addition to its effect on learning, the *for* polymorphism influences a number of other behavioral and physiological traits of ecological relevance (19, 22, 55, 56). It also affects larval competitive ability in a density-dependent manner, whereby high population density favors the for^R allele and low density favors the for^s allele (22). Furthermore, under some circumstances, negative frequency-dependent selection seems to favor whichever of the two alleles is currently rare (23), likely contributing to the maintenance of this polymorphism in nature. Thus, the overall force of selection acting on the *for* alleles will reflect the aggregate impact of their manifold pleiotropic effects on survival and reproduction. If such a high degree of pleiotropy were typical of natural alleles affecting learning, there would be two important consequences for evolution of cognitive traits. First, evolutionary changes in learning ability would be associated with changes in other ecologically relevant traits. Second, improved learning or memory might evolve as a byproduct of natural selection on other traits rather than because of fitness advantages of learning itself.

Materials and Methods

Fly Strains. We used isogenic strains homozygous for for^R, for^s, and for^{s2}, which were maintained at the University of Toronto until a few generations before the experiments. The for^R (rover) and for^s (sitter) strains carry natural allelic variants of the *for* gene, which is located on chromosome 2. To control for genetic background, they have coisogenic third chromosomes (originating from the rover strain) and shared X-chromosomes. The for^{s2} strain is a sitter mutant generated on a rover for^R genetic background (20), such that for^{s2} differs from for^R only in their alleles at *for*. The heads of for^s and for^{s2} flies have significantly lower PKG enzyme activity than those of the for^R strain (18).

GAL4 Lines. All of the UAS and GAL4 constructs used for the behavioral assays were crossed into a *white*^{1} (w^1) sitter (for^s) genetic background. Specifically, the second-chromosome GAL4 driver lines w^1;for^s *201Y-GAL4*, w^1;for^s *30Y-GAL4* and w^1;for^s *c739-GAL4* were obtained by backcrossing the corresponding GAL4 elements into the w^1;for^s background for nine generations. For transgenic expression of *for*, a w^1;for^s;*UAS-forT2* line was made by using a *foraging dg2-T2* cDNA construct from D. Kalderon (27, 57). To express *forT2* we crossed the w^1;for^s;*UAS-forT2* line to an appropriate GAL4 line (28) and tested the progeny of these crosses. The progeny of a cross between each of the GAL4 lines to w^1;for^s and of the *UAS-forT2* line to w^1;for^s were used as negative controls.

Experimental Conditions. All flies were cultured on a standard cornmeal medium. Flies were bred, conditioned, and tested at 25°C. When testing was performed 24 h after conditioning, the flies spent the 24 h between conditioning and testing at 18°C; this temperature is more conducive to long-term memory formation and/or maintenance (26). The flies to be assayed for learning were never anesthetized.

Learning Assays. We used a classical conditioning assay in which flies associate an odor with mechanical shock (3). Conditioning and memory tests were performed on samples of ≈50 adult flies (sexes mixed), aged 3–5 days. Three conditioning protocols were used: (*i*) a single conditioning cycle, (*ii*) five conditioning cycles separated by 20-min intervals (spaced protocol), and (*iii*) five conditioning cycles immediately after one another (massed protocol). In each conditioning cycle, flies were first exposed for 30 s to one odorant and simultaneously subject to a mechanical shock (2,000-rpm vibration pulses of 1-s duration, delivered every 5 s by a test tube shaker). This period was followed by a 60-s rest period (no odor and no shock). Then, for 30 s, another odorant was delivered without a shock. The conditioning round ended with a second rest period of 60 s. 3-octanol and 4-methylcyclohexanol (both 0.6 ml/l of paraffin) were used as odorants.

We tested 15-min or 24-h memory retention. Flies were transferred to the choice point of a T-maze, at which time they were exposed to two converging currents of air, one carrying octanol and the other methylcyclohexanol, and allowed to choose between the two odors for 60 s. The count of flies in each arm of the maze after 60 s was used to calculate the proportion of flies choosing (i.e., moving toward) octanol. Flies that remained in the entry chamber of the T-maze were excluded from this calculation; their number did not differ significantly in any assay among the *for* strains (ANOVA, all $P > 0.25$) or among the transgenic lines (all $P > 0.3$).

For the analysis, a unit of replication consisted of two samples of 50 flies. One sample was conditioned to avoid octanol and the other to avoid methylcyclohexanol; a single value of the memory index was calculated as the difference in the proportion of flies choosing octanol between these two samples. For statistical comparison of the memory indices (but not for graphical representation of the data), all proportions were arcsine-square-root-transformed before the analysis (58). To compare the memory index among lines, we used an ANOVA. The assays were carried out over several days, in blocks consisting of one replicate of each line. A block effect was thus included in the ANOVA as a random factor (58). To test for differences in the memory index between the GAL4 × UAS cross and corresponding control crosses (GAL4 × w^1;for^s and w^1;for^s × UAS), we used a planned contrast within the ANOVA framework (58).

Unconditioned Response to Odors. Both odorants used in the learning assays are moderately repulsive to naive flies. To exclude, as a confounding factor, the differences among the fly lines in the unconditioned response to the odorants, we assayed their avoidance response to octanol or methylcyclohexanol in the absence of conditioning. Groups of 50 unconditioned flies were tested in the T-maze assay for their choice between an odorant dissolved in paraffin vs. paraffin alone. An odor-avoidance index was calculated as the proportion of flies avoiding the odorant (i.e., moving toward paraffin only; flies that remained in the central chamber of the T-maze were excluded from this calculation). These proportions were arcsine-square-root-transformed for the analysis.

Immunohistochemistry. ***Native FOR expression.*** Whole-mount adult brains of 3- to 7-day-old WT flies (reared at 25°C) were dissected in PBS and fixed for 50 min in 4% paraformaldehyde. Fixed tissues were washed several times with 0.5% Triton-X 100/PBS (PBT) and incubated in blocking solution (10% normal goat serum/0.1% bovine serum albumen/PBT) for several hours. For immunohistochemical study of endogenous FOR expression, tissues treated with blocking solution were incubated at 4°C for 48 h in 1:100 dilution of FOR antiserum made against all of the FOR isoforms (27). Tissues were then washed several times with PBT and incubated with fluorescently labeled secondary antibody (Cy2-conjugated anti-guinea pig IgG, 1:100 dilution) (Jackson ImmunoResearch, West Grove, PA). Fluorescently labeled tissues were then examined with an LSM 510 laser-scanning microscope (Zeiss, Oberkochen, Germany). Images were adjusted for levels and contrast in Adobe Photoshop (Adobe, San Jose, CA).

Mushroom body GAL4 expression patterns. To visualize the expression patterns of the mushroom body GAL4 drivers, each of the three GAL4 strains (all in a w^1;for^s genetic background, as described above) were crossed to a UAS-reporter line (*UAS-mCD8-GFP*, Bloomington *Drosophila* Stock Center) to generate adult flies carrying both the GAL4 driver and UAS-mCD8GFP. Brains of those flies were dissected and prepared as above and were incubated overnight at 4°C in rat anti-CD8 antibody (1:100, Caltag, Carlsbad, CA). Tissues were then washed several times, incubated in secondary antibody, and imaged as above.

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1. Dukas R (2004) *Annu Rev Ecol Evol Syst* 35:347–374.
2. Mery F, Kawecki TJ (2003) *Proc R Soc London B* 270:2465–2469.
3. Mery F, Kawecki TJ (2005) *Science* 308:1148–1148.
4. Tryon RC (1940) *Yk Natl Soc Stud Educ* 39:111–119.
5. McGuire TR, Tully T (1987) *Behav Genet* 17:97–107.
6. Lofdahl KL, Holliday M, Hirsch J (1992) *J Comp Psychol* 106:172–183.
7. Mery F, Kawecki TJ (2002) *Proc Natl Acad Sci USA* 99:14274–14279.
8. Wadell S, Quinn WG (2001) *Annu Rev Neurosci* 24:1283–1309.
9. Dubnau J, Chiang A-S, Tully T (2003) *J Neurobiol* 54:238–253.
10. Davis RL (2005) *Annu Rev Neurosci* 28:275–302.
11. Heisenberg M (2003) *Nat Rev Neurosci* 4:266–275.
12. Morrison GE, van der Kooy D (2001) *Behav Neurosci* 115:640–649.
13. Kuhara A, Mori I (2006) *J Neurosci* 26:9355–9364.
14. Powell CM (2006) *Neurobiol Learn Mem* 85:2–15.
15. Fanselow MS, Poulos AM (2005) *Annu Rev Psychol* 56:207–234.
16. Papassotiropoulos A, Stephan DA, Huentelman MJ, Hoerndli FJ, Craig DW, Pearson JV, Huynh KD, Brunner F, Corneveaux J, Osborne D, *et al.* (2006) *Science* 314:475–478.
17. de Quervain DJF, Papassotiropoulos A (2006) *Proc Natl Acad Sci USA* 103:4270–4274.
18. Osborne KA, Robichon A, Burgess E, Butland S, Shaw RA, Coulthard A, Pereira HS, Greenspan RJ, Sokolowski MB (1997) *Science* 277:834–836.
19. Sokolowski MB (2001) *Nat Rev Genet* 2:879–890.
20. Pereira HS, Sokolowski MB (1993) *Proc Natl Acad Sci USA* 90:5044–5046.
21. Scheiner R, Sokolowski MB, Erber J (2004) *Learn Mem* 11:303–311.
22. Sokolowski MB, Pereira HS, Hughes K (1997) *Proc Natl Acad Sci USA* 94:7373–7377.
23. Fitzpatrick MJ, Feder E, Rowe L, Sokolowski MB (2007) *Nature* 447:210–212.
24. Feil R, Hofmann F, Kleppisch T (2005) *Rev Neurosci* 16:23–41.
25. Hofmann F, Feil R, Kleppisch T, Schlossmann J (2006) *Physiol Rev* 86:1–23.
26. Tully T, Preat T, Boynton SC, Del Vecchio M (1994) *Cell* 79:35–47.
27. Belay AT, Scheiner R, So AKC, Douglas SJ, Chakaborty-Chatterjee M, Levine JD, Sokolowski MB (2007) *J Comp Neurol*, in press.
28. Brand AH, Perrimon N (1993) *Development (Cambridge, UK)* 118:401–415.
29. Zars T, Fischer M, Schulz R, Heisenberg M (2003) *Science* 288:672–675.
30. Akalal DBG, Wilson CF, Zong L, Tanaka NK, Ito K, Davis RL (2006) *Learn Mem* 13:659–668.
31. Krashes MJ, Keene AC, Leung B, Armstrong JD, Waddell S (2007) *Neuron* 53:103–115.
32. Feil R, Hartmann J, Luo CD, Wolfsgruber W, Schilling K, Feil S, Barski JJ, Meyer M, Konnerth A, De Zeeuw CI, Hofmann F (2003) *J Cell Biol* 163:295–302.
33. Kleppisch T, Wolfsgruber W, Feil S, Allmann R, Wotjak CT, Goebbels S, Nave KA, Hofmann F, Feil R (2003) *J Neurosci* 23:6005–6012.
34. Chien WL, Liang KC, Teng CM, Kuo SC, Lee FY, Fu WM (2005) *Eur J Neurosci* 21:1679–1688.
35. Edwards TM, Rickard NS, Ng KT (2002) *Neurobiol Learn Mem* 77:313–326.
36. Matsumoto Y, Unoki S, Aonuma H, Mizunami M (2006) *Learn Mem* 13:35–44.
37. Mery F, Pont J, Preat T, Kawecki TJ (2007) *Physiol Biochem Zool* 80:399–405.
38. Pascual A, Preat T (2001) *Science* 294:1115–1117.
39. Yu D, Akalal DBG, Davis RL (2006) *Neuron* 52:845–855.
40. Papaj DR, Prokopy RJ (1989) *Annu Rev Entomol* 34:315–350.
41. Dukas R (2006) *Anim Biol* 56:125–141.
42. De Boer JG, Dicke M (2006) *Anim Biol* 56:143–155.
43. Vet LEM, Groenewold AW (1990) *J Chem Ecol* 16:3119–3135.
44. Dukas R, Bernays EA (2000) *Proc Natl Acad Sci USA* 97:2637–2640.
45. Coolen I, Dangles O, Casas J (2005) *Curr Biol* 15:1931–1935.
46. Menzel R, Muller U (1996) *Annu Rev Neurosci* 19:379–404.
47. Dukas R (1998) *Behav Ecol Sociobiol* 19:195–200.
48. Dukas R (2004) *Behav Ecol* 15:695–698.
49. Reif M, Linsenmair KE, Heisenberg M (2002) *Anim Behav* 63:143–155.
50. Dukas R (2005) *Anim Behav* 69:1203–1209.
51. Mery F, Kawecki TJ (2004) *Anim Behav* 68:589–598.
52. Chabaud MA, Devaud JM, Pham-Delegue MH, Preat T, Kaiser L (2006) *J Comp Physiol* 192:1335–1348.
53. Kaun KR, Hendel T, Gerber B, Sokolowski MB (2007) *Learn Mem*, 14:342–349.
54. Shettleworth SJ (1999) *Cognition, Evolution, and Behavior* (Oxford Univ Press, Oxford).
55. Kraaijeveld AR, Van Alphen JJM (1995) *J Insect Behav* 8:305–314.
56. Engel JE, Xie XJ, Sokolowski MB, Wu CF (2000) *Learn Mem* 7:341–352.
57. Kalderon D, Rubin GM (1989) *J Biol Chem* 264:10738–10748.
58. Sokal RR, Rohlf FJ (1995) *Biometry: The Principles and Practice of Statistics in Biological Research* (Freeman, New York), 3rd Ed.

Fig. 4. *for* and the response to odors. Mean (±SEM) avoidance of octanol (*A* and *B*) and methylcyclohexanol (*C* and *D*) of naïve flies of the rover (for^R) and sitter (for^s, for^{s2}) genotypes (*A* and *C*) and the transgenic lines (*B* and *D*). Odor avoidance was assayed as the proportion of flies moving away from an odorant when given a choice between the odorant and air. Octanol was avoided more strongly than MCH (mean avoidance index 0.86 versus 0.83, $F_{1,95} = 10.2$, $P = 0.002$), but there were no significant differences among lines ($F_{9,95} = 1.2$, $P = 0.31$) and no line × odor interaction ($F_{9,95} = 1.6$, $P = 0.10$). $n = 6$ per strain and odorant.